PHYTOCHEMICAL AND ANTITUSSIVE INVESTIGATION OF BARK EXTRACTS OF ACACIA NILOTICA

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ABSTRACT: The main objective of present work is phytochemical and antitussive investigation of bark Extracts of Acacia Nilotica. Antitussives are remedies that alleviate coughing. Some antitussives work by soothing irritability (respiratory demulcents); others are claimed to relieve coughs at source, by removing congestive mucus or other mobile provocations (expectorants). Although the centrally acting opioids still remain the antitussive drug of choice for decades, they possess side effects such as sedation and gastrointestinal symptoms. The ethyl acetate and methanolic extracts of the bark of Acacia nilotica. were evaluated for antitussive action in mice using ammonium liquor induced cough and sulfur dioxide induced cough models at two different dose levels (250 and 500 mg/kg, po). The results indicate that the bark extract of Acacianilotica demonstrated antitussive effect in in vivo experimental models by prolonging the latency period of coughing and also reducing the frequency of coughing bouts in mice. The dose of 500 mg/kg of the methanolic extract was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models.

Keywords: Antitussive activity, Acacianilotica, Acacia bark, qualitative phytochemical analysis, plant extracts, cough suppressant activity, flavonoids

INTRODUCTION: Plant and other natural products have been in use for the human sufferings from time immemorial. The search for new chemical entities obtained by screening natural sources such as plant extracts and microbial fermentation had led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. Today, higher plants continue to retain their historical significance as important sources of novel compounds useful either directly as medicinal agents or as lead compounds for synthetic/semi synthetic structural modification/optimization or biochemical/pharmacological probes. Natural products, in particular herbs, have been used for the treatment of various diseases for thousands of years. Terrestrial plants are used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs are developed from them. The primary written records on the medicative uses of plants appeared in concerning 2600 BC from the Sumerians and Akkaidians. The “Ebers Papyrus”, the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 B.C. The Chinese Materia Medica, which describes more than 600 medicinal plants, has been well

documented with the first record dating from about 1100 B.C [2]. Documentation of the Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC [3]. The use of medicinal plants was compiled in Ayurveda, which listed more than 8000 herbal remedies. India is one of the world's twelve leading biodiversity centers with the presence of over 45,000 different plant species. Of these, about15,000-20,000plantshavemedicinalpropertiesofwhichonly about7000-7500are being used by traditional practitioners. Siddha system of medicine uses around 600, Ayurveda700, Amchi 600, Unani 700 and modern medicine about 30 plant species. Projection is being made that next to information technology, herbal technology will be India's biggest revenue earner. The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician(100 A.D.), described in hiswork "De Materia Medica" more than 600 medicinal plants. The World Health Organization estimates that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care. Cancer is a major public health burden in both developed and developing countries. It was calculated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002.Cancer is the second leading cause of death in the United States, where everyone in four deaths is due to cancer. Plants have long been used in the treatment of cancer. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. In recent years, there is amazing interest in the possible role of nutrition in prevention of disease. In this context, antioxidants particularly derived from natural sources such as Indian medicinal plants and herbal drugs derived from them require special attention. Antioxidants neutralize the toxic and'volatile'free radicals. Antioxidants have many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy. In biological systems oxygen gives rise to a large number of free radicals and other reactive species collectively known as 'reactive oxygen species' (ROS). Another group of reactive species are termed as 'reactive nitrogen species' (RNS). In a normal healthy human, the generation of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when the humans get exposed to adverse physiochemical, environmental or pathological agents this delicately maintained balance is shifted in favour of pro-oxidants resulting in oxidative stress. Cellular damage induced by oxidative stress has been implicated in the etiology of a large number (>100) of human diseases as well as the process of ageing. Various antioxidants may prevent and/or improve diseased states. These include the intracellular antioxidant enzymes and the dietary or oral supplements in the form of vitamin C, vitamin E, β-carotene, zinc and selenium. Antioxidants also can act at different levels of protection such as prevention, interception and repair. Indian medicinal plants provide a rich source of antioxidants. A cough is a symptom of a variety of respiratory and non-respiratory conditions which could be both mild and serious in nature and can be described as "a forced expulsive man oeuvre against a closed glottis that are associated with a characteristic sound or sounds". Causes of cough can range from a common cold to malignancy and pharmacists should be able to distinguish between a cough not resulting from a serious pathology and one which could be the underlying symptom of a potentially critical condition. Studies have shown that the reporting of cough is more prevalent in females than males, possibly due to an increased sensitivity of cough reflex in women. An acute cough is often the result of an upper

respiratory tract infection and although distressing it is usually self-limiting and does not require any medical intervention. An acute cough is defined as one lasting less than three weeks. When a patient presents with an acute cough the pharmacist should still enquire about haemoptysis, the possibility of an inhaled foreign body and prominent systemic illness since cough can be the first indication of an underlying serious condition. Common causes of an acute cough are upper respiratory tract infections, exacerbations of chronic obstructive pulmonary disease (COPD), allergic rhinitis, and rhinitis due to environmental irritants and asthma which is not well controlled. A chronic cough is one which lasts more than 8 weeks and accounts for one-tenth of respiratory referrals to secondary care. Cough lasting between three to eight weeks is defined as subacute cough. In general, most chronic coughs are dry or minimally productive in nature although some pathologies result in a chronic cough with the presence of significant sputum production. Although chronic cough is perceived as trivial, it can be a disabling symptom associated with significant morbidity. Chronic cough would have started off as an acute cough and therefore it is important to identify the exact duration of this symptom so as to narrow the list of possible causes.

Some of the most common causes of chronic cough in immune-competent patients are asthma, gastro oesophageal reflux disease, chronic bronchitis due to cigarette smoking and other irritants, bronchi ectasis, eosinophilic bronchitis, postnasal-drip syndrome from conditions of the nose and sinuses, or the use of an angiotensin-converting-enzyme (ACE) inhibitor. Some physical symptoms can occur as a consequence of cough, such as musculoskeletal pains, hoarseness, stress incontinence, blackouts and vomiting. Therefore, patients presenting with an unknown cause of the afore-mentioned physical symptoms should be asked about a history of coughing.

Common causes of cough

The following are just a few of the most common causes of cough:

1. Angiotensin-Converting-Enzyme (ACE) Inhibitor induced cough.
2. Asthma, COPD, Acute bronchitis, Chronic bronchitis and Eosinophilic bronchitis.
3. Environment and smoking induced cough.
4. Gastro-oesophageal reflux disease, Heart failure, Malignancy & Upper respiratory tract infections.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL: Stem bark of the plant under investigation i.e., Acacianilotica was collected from near by areas of Bhopal (M.P), India in the month of May 2019. The taxonomical identification and authentication of the plant material was done at Saifia science college Bhopal (Affiliated to barkatullah university Bhopal, India).The specimen of plant sample has been submitted and preserved in the herbarium of the institute.

Chemicals: Petroleum ether, Chloroform, Methanol, Ethyl acetate, Glacial acetic acid, Acetone, Formic acid, Benzene, N-propanol, Ethanol, Tween-80, Dimethylsulphoxide (DMSO), Glycerine, conc. Sulphuric acid, Hydrochloric acid, N-Butanol,Dichloromethane,n-hexane,pyridine,toluene,xylene,anisaldehyde, α-naphthol, bismuth carbonate, calcium chloride, copper sulphate, Ferric chloride, Folin's reagent, Iodine, Lead acetate, Magnesium chloride, Mercuric chloride, Ninhydrin, Nitric acid, Phloroglucinol, Potassium iodide, Potassium Dichromate, Potassium sodium Tartarate, Ruthenium red, Safranine, Sodium acetate, Sodium iodide, Sodium hydroxide, Sodium nitroprusside. All the chemicals (S.D Fine Chemicals Pvt. Ltd. Mumbai) were purchased from local supplier and were of analytical grade.

Instruments and Equipments

- **Digital balance**(Wensar):A highly sensitive instrument used for weighing substances to the milligram (0.001 g) level was used.
- **Refrigerator & Freezer: A** double door refrigerator (Whirlpool) present in the lab was used to fulfill both purposes of a refrigerator and a freezer.
- **Water Bath (Navyug):** In lab rectangular digitally controlled serological water bath was used.
- **Soxhlet Apparatus (ASGI):** It was used for hot continuous extraction of the plant material using various solvents.
- **Other Materials Used:** Micropipettes & tips, Micro centrifuge tubes, Beaker, Flask, Micro centrifuge tubes stand, Aluminum foil, Spatula, Butter paper, Cotton, Forceps, Tissue paper, range of glassware & lab ware.

PHARMACOGNOSTIC STUDIES: The pharmacognostical evaluation of plants is the prelude stride in the standardization of plant drugs.

Preparation of Acacia nilotica powder

The stem bark of Acacia nilotica was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in a labeled air tight container for further studies.

Physicochemical studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of Acacia nilotica.

Result and Discussion

Macroscopic and microscopic evaluation: organoleptic evaluation represents observing of those properties of materials for which sense organs can be used. It there by defines some specific characteristics of the material which can be considered as a first step towards establishing the identity and degree of purity of the material. The organoleptic parameters (color, odour, taste and texture) were evaluated and are presented in Table 1.

Table 1: Organoleptic Characters of Acacia nilotica

Plant parts	Color	Odour	Taste
Flowers	Yellow	Sweet scented	Not evaluated
Leaves	Green colour	Nil	Slightly Bitter
Stem	Brown color	Nil	Slightly Bitter

It was evident from the macroscopic evaluation of the plant material that the yellow colored flowers have round head and velvety texture, the bipinnate leaves with hairy axis belonged to the plant Acacia nilotica. A similar macroscopic evaluation for the plant was also reported by Lawrence et al. The microscopic features of the bark were observed under a microscope and revealed the presence of thick epidermal cells.

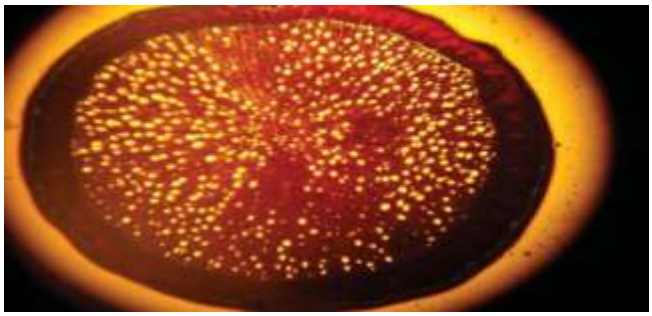

Figure 1: T.S. of Acacia nilotica bark exhibiting thick epidermis and xylem cells

The powder microscopy revealed the presence of starch granules and xylem vessels in the dried powder of the bark (Figure 2 and 3).

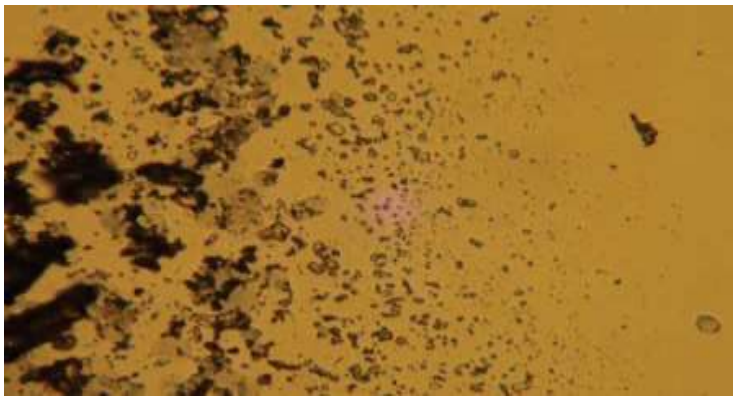

Figure 2: Starch granules visible under light microscope in the bark powder

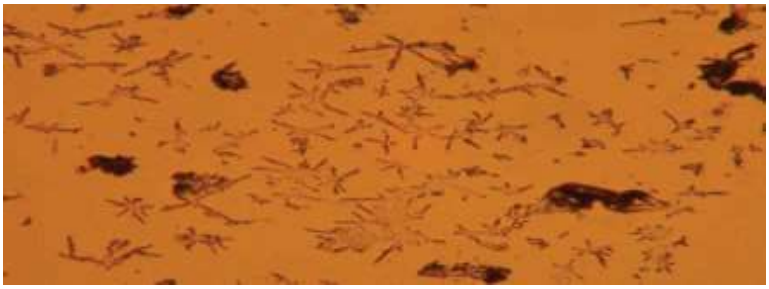

Figure 3: Xylem vessels visible under light microscope in the bark powder

Determination of Percentage yield

The crude extracts so obtained after each of the successive soxhlet extraction process were concentrated on water bath by evaporation of the solvents to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of the bark extracts obtained from different solvents is presented in Table 2.

Table 2: Results of Percentage yield of Bark Extracts

Plant Name	Percentage yield				
	Pet.Ether	Chloroform	Ethylacetate	Ethanol	Water
Acacia nilotica	1.8	1.23	2.03	4.43	6.9

Estimation of Physical Parameters

Determination of ash value: The ash values are useful to determine the quality and purity of the crude drugs.

Table 3: Ash value of plant material

S. No.	Total ash (%w/w)	Acid Insoluble Ash (%w/w)	Water soluble Ash (%w/w)
1	10.2%	6.8%	2.3%

Determination of extractive values: Extractive values of crude drugs are useful for the evaluation of crude drugs.

Table 4: Extractive value of plant material

S. No.	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)	Ether soluble extractive value (% w/w)
1	15.1%	9.8%	11.1%

Loss on Drying

This parameter is used to determine the amount of moisture present in a particular sample. The amount of moisture affects the powder characteristics as it may cause the growthof microbes. The total moisture content present in the dried bark was foundto be 2.35%.

Phytochemical Evaluation

A small portion of the dried extracts were subjected to the phytochemical for presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoid separately for extracts of all samples.Smallamountofeachextractissuitablyresuspendedintothesteriledistilledwater to make the concentration of 1 mg per ml. The result of qualitative phytochemical analysis is depicted in the Table 7.

Table 5: Phytochemical analysis of different bark extracts

Chemical Tests	Pet. Ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Aqueous extract
Alkaloids					
Mayer's reagent	-	-	-	-	+
Hager's reagent	-	-	-	-	+
Wagner's reagent	-	-	-	-	+
Dragendorff's Reagent	-	-	-	-	+
Glycosides(+Ve)					
Baljet test	-	+	+	+	-

Legal's test	-	-	+	+	-
Keller-Killani	-	+	+	-	-
Phenols/Tannins					
Ferric chloride	-	-	+	+	+
Gelatin Solution	-	-	+	+	+
Lead acetate test	-	-	+	+	+
Flavonoids					
$FeCl_3$ test	-	-	+	+	-
Alkaline reagent test	-	-	+	+	-
Shinoda test	-	-	+	+	-
Saponins					
Foam test	-	-	-	-	+
Hemolytic test	-	-	-	-	+
Lead acetate	-	-	-	-	+
Fixed oil/Fats					
Spot	+	-	-	-	-
Saponification	+	-	-	-	-
Gums & Mucilage					
Water	-	-	-	-	-
Carbohydrates					
Molisch test	-	-	+	+	-
Fehling's solution test	-	-	+	+	-
Benedict's test	-	-	-	+	-

(+) Indicates'Presence';(-) Indicates'Absence'

The results obtained from phytochemical testing of the successive solvent extracts of the barks of Acacianilotica revealed the presence of alkaloids in the aqueous extracts. Phenols and tannins were obtained in the ethyl acetate, methanolic and aqueous extracts where as flavonoids were obtained in the ethyl acetate extract and methanolic

extract. Saponins were tested positive only in the aqueous extracts where as glycosides were detected in the chloroform, ethyl acetate and methanolic extracts. Carbohydrates were found in the ethyl acetate and methanolic extracts of the bark. In their study Lawrence etal also found presence of glycoside, Flavonoids, phenols and glycosides and carbohydrates in the bark of Acacia nilotica.

Antitussive screening of extracts: The evaluation of antitussive activity in animal can be done by inducing cough by mechanical stimulus, electrical stimulus, and chemical stimulus. In the present study chemical induction of cough was performed and the effect of the bark extract on cough bouts was recorded. A cough bout response to a given stimulus varies from animal to animal but fairly reproducible if we repeat the measurements within the same animals. So, low or high cough bout threshold in animals were not entertained for further studies. The frequency of cough bouts was observed for all animal groups at 1hr after administration of standard drug aswell as Acacia nilotica bark extracts. The percentage inhibition of frequency of cough bout was calculated by the formula-

$$\% \text{ percentage inhibition of frequency of cough} = [(Ca - Ta)/Ca] X100$$

Where,

Ta= Frequency of cough bout in treated animal,

Ca= Frequency of cough bout in control group

The time interval between exposure to ammonia hydroxide or SO_2 and appearance of cough bouts is called as the cough latency period. It is a measure of the potency and efficacy of the drug under study. on the basis of the qualitative phytochemical analysis results it was evident that the major portions of the phytochemicals especially tannins, saponins, Flavonoids and Triterpenoids were present in the ethyl acetate and methanolic extracts of the bark of Acacia nilotica. It was therefore decided to evaluate these two extracts for antitussive action in mice using two different models that used chemical induction of cough. The results obtained fromthe study against ammonium liquor induced cough and sulfur dioxide induced cough in mice.

Table 6: Antitussive effect of Acacianilotica extract against Ammonium liquor induced coughing

Group	Treatment	Dose (mg/kg,p.o)	Latency Period (Sec)	Number of coughing bouts	Percent Inhibition of cough bout
I	Control	10	11.22±2.01	90.5±1.41	-
II	Ethyl acetate extract	250	14.02±2.36*	41.06± 4.36*	54.6
III	Ethyl acetate extract	500	22.44± 3.39***	21.24± 2.71***	76.5
IV	Methanolic extract	250	19.13± 2.45**	37.79± 4.35*	58.2
V	Methanolic extract	500	27.44± 3.33***	19.33± 2.62***	78.6
VI	Codeine phosphate	10	36.39± 3.65***	10.08± 1.21***	88.9

Values expressed as mean±SEM (n=6).*P<0.05,**P<0.01,and***P<0.001for comparison of treated groups with control

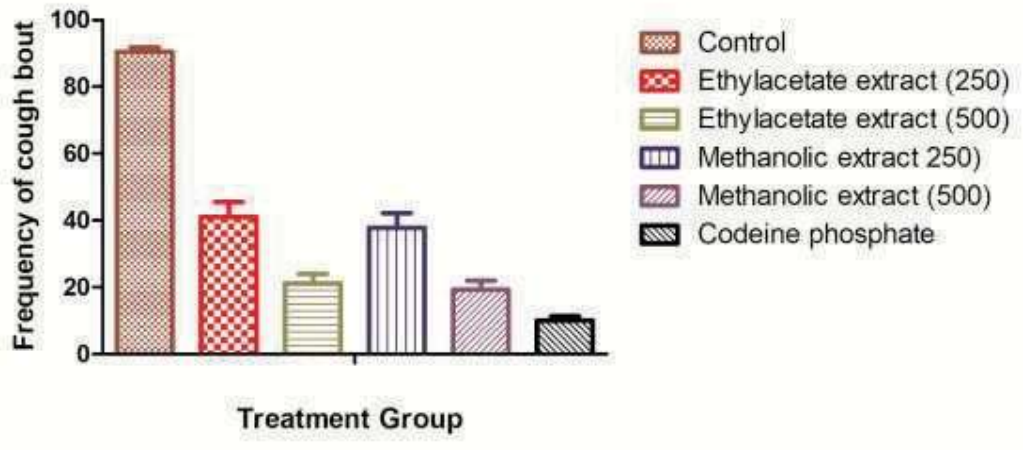

Figure 4: Frequency of coughing bouts against ammonium liquor induced cough

Table 7: Antitussive effect of Acacianilotica extract against sulfurdioxide induced coughing

Group	Treatment	Dose (mg/kg,p.o)	Latency Period (Sec)	Number of coughing bouts	Percent Inhibition of cough bout
I	Control	10	11.22±2.01	76.73±3.32	-
II	Ethyl acetate extract	250	14.02±2.36*	29.33± 1.19*	61.8
III	Ethyl acetate extract	500	22.44± 3.39***	22.47± 2.21**	70.7
IV	Methanolic extract	250	19.13± 2.45**	27.14± 2.44***	64.6
V	Methanolic extract	500	27.44± 3.33***	18.66± 1.17***	75.7
VI	Codeine phosphate	10	36.39± 3.65***	10.78± 1.44***	86

Values expressed as mean±SEM(n=6).*P<0.05,**P<0.01,and***P<0.001 for comparison of treated groups with control

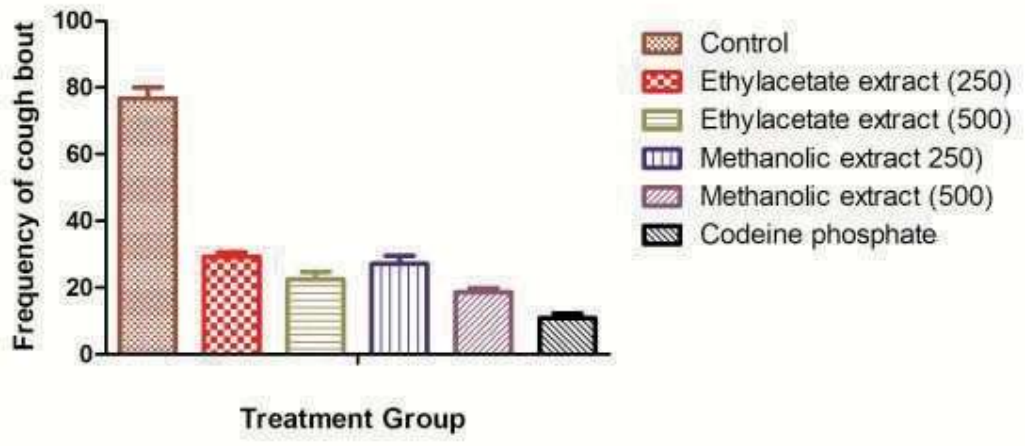

Figure 5: Frequency of coughing bouts against sulfurdioxide induced cough

It was obvious from the results that the antitussive action was much more significant in the methanolic extract as compared to the ethyl acetate extract. The dose of 500 mg/kg of the methanolic extract was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models.

The results indicate that the bark extract of Acacianilotica demonstrated antitussive effect in in vivo experimental models by prolonging the latency period of coughing and also reducing the frequency

of coughing bouts in mice.The increase in latency as well as the decrease in the frequency of coughing bouts occurred in a dose dependent fashion in both the extracts (Figure 4)

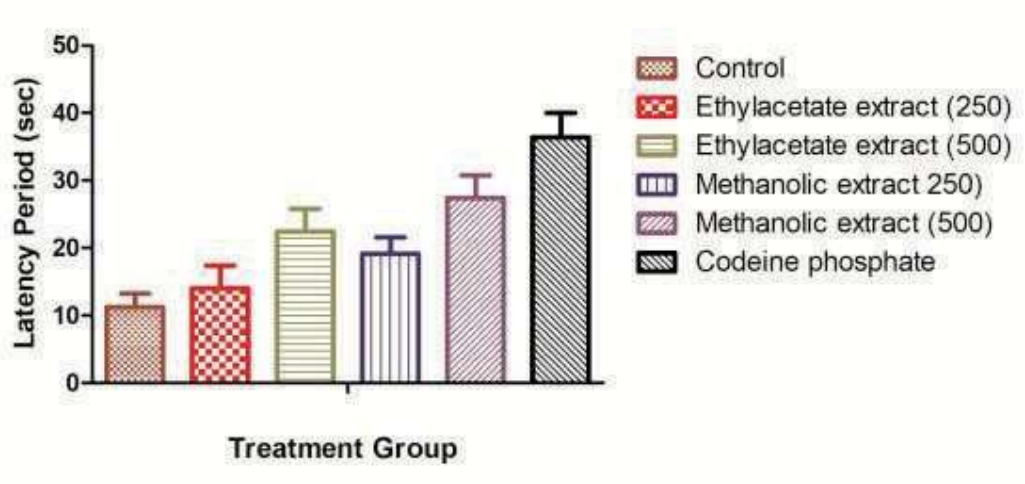

Figure 6: Observed latency period for coughing bouts

Thus it was observed that oral administration of the extract reduced the frequency of cough in a dose related fashion which was statistically significant when compared with the control group

SUMMARY AND CONCLUSION: As it was evident from the review of the literature that the bark of Acacia nilotica was scientifically the least explored part,in the present work it was therefore envisioned to explore the folkloric reported cough suppressant activity of the bark of the plant. The plant material was collected from the local places of Bhopal in the month of June and was authenticated by macroscopic and microscopic characterization. The macroscopic characters evaluated included the shape, size, taste, smell, texture and color of the different parts of the plant including leaves, stem, bark and flowers. The macroscopic evaluation revealed yellow colored velvety flowers, bipinnate leaves with hairy axis and reddish brown bark.The microscopic evaluation of the transverse section of the bark revealed thick epidermis where as the powder characteristics exhibited xylem vessels and starch granules. The total ash value of the bark was found to be 10.2% while the acid insoluble as was 6.8% and the water soluble ash was found to be 2.3%. The alcohol soluble extractive value was found to be 15.1% w/w, water soluble extractives 9.8% and ether soluble extractive value was found to be 11.1% w/w. Successive solvent extraction of the bark powder was carried out in various solvents including petroleum ether, chloroform, ethyl acetate, methanol and water and the qualitative phytochemical tests were performed on each extract for determination of the presence of alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrates, steroids and other phytochemicals. The results obtained from phytochemical testing of the successive solvent extracts of the barks of Acacia nilotica revealed the presence of alkaloids in the aqueous extracts. Phenols and tannins were obtained in the ethylacetate, methanolic and aqueous extracts whereas flavonoids were obtained in the ethyl acetate extract and methanolic extract. Saponins were tested positive only in the aqueous extracts where as glycosides were detected in the chloroform, ethylacetate and methanolic extracts.Carbohydrates were found in the ethyl acetate and methanolic extracts of the bark. On the basis of the

qualitative phytochemical analysis results it was evident that the major portions of the phytochemicals especially tannins, saponins, flavonoids and triterpenoids were present in the ethyl acetate and methanolic extracts of the bark of Acacia nilotica. These two extracts were evaluated for antitussive action in mice using ammonium liquor induced cough and sulfur dioxide induced cough models at two different dose levels (250 and 500 mg/kg, po).The results indicate that the bark extract of Acacia nilotica demonstrated antitussive effect inin vivo experimental models by prolonging the latency period of coughing and also reducing the frequency ofcoughing bouts in mice.The increase in latency as well as the decrease in the frequency of coughing bouts occurred in a dose dependent fashion in both the extracts. It was obvious from the results that the antitussivea ction was much more significant in the methanolic extract as compared to the ethyl acetate extract. The dose of 500 mg/kg of the methanolic extract was able to suppress the coughing bouts comparable to the standard drug codeine phosphate in the both the experimental models. It can be concluded form the study that as reported in the folkloric literature, the bark extract of plant Acacia nilotica has significant anti-cough effect in experimentally induced cough reflex in mice like the standard drug (codeine phosphate). It can be assumed that the extract might be acting via the central nervous system. Further work related to the isolation and characterization of the active constituents as well as evaluation of the mechanism of antitussive effect will be carried out in our laboratory in the near future

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